



*Press Release, December 19, 2017*

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## Visualizing single molecules in whole cells with a new spin

### A new imaging method could put super-resolution microscopy within reach of most biologists

Cell biologists traditionally use fluorescent dyes to label and visualize cells and the molecules within them under a microscope. With different super-resolution microscopy methods, they can even light up single molecules and see their complex interactions with one another. However, the microscopy hardware required to do this is highly specialized, expensive, and requires operators to have unique skills; hence, such microscopes are relatively rare in laboratories around the world.

Ralf Jungmann, Ph.D., head of the research group "Molecular Imaging and Bionanotechnology" at Max Planck Institute (MPI) of Biochemistry in Germany, Professor of Biochemistry at the Ludwig Maximilian University (LMU) and alumnus of the Wyss Institute and the Wyss Institute Core Faculty member Peng Yin, Ph.D. have been developing DNA-PAINT, a powerful molecular imaging technology that involves transient DNA-DNA interactions to accurately localize fluorescent dyes with super-resolution. However, although the researchers demonstrated DNA-PAINT's potential by visualizing single biomolecules such as proteins in fixed cells at a fixed close distance, the technology could not yet investigate molecules deep inside of cells.

Now, Jungmann's and Yin's teams jointly report a solution to overcome this limitation. In their new study, they adapted DNA-PAINT technology to confocal microscopes, which are widely used by researchers in cell biology laboratories to image whole cells and thicker tissues at lower resolution. The MPI/Wyss Institute team demonstrates that the method can visualize a variety of different molecules, including combinations of different proteins, RNAs, and DNA throughout the entire depth of whole cells at super-resolution. Published in *Nature Communications*, the approach could open the door for detailed single-molecule localization studies in many areas of cell research.

The DNA-PAINT approach attaches a DNA "anchor strand" to the molecule of interest. Then a dye-labeled DNA "imager strand" with a complementary sequence transiently attaches to the anchor and produces a fluorescent signal, which occurs as a defined blinking event at single molecular sites. Because this "blinking frequency" is precisely tunable, molecules that are only nanometers apart from each other can be distinguished — at the higher resolution end of super-resolution.





“Our new approach, SDC-PAINT, integrates the versatile super-resolution capabilities of DNA-PAINT with the optical sectioning features of confocal microscopes. We thus created the means to explore the entire depth of a cell, and to visualize the molecules within it at the nanometer scale,” said Jungmann. The team mapped out the presence of different combinations of proteins within whole cells, and then went beyond that. “By diversifying our labeling approaches, we also visualized different types of individual biomolecules in the chromosome-containing nucleus, including sequences in the DNA, proteins bound to DNA or the membrane that encloses the nucleus, as well as nuclear RNAs,” adds Yin, who is also co-leader of the Wyss Institute’s Molecular Robotics Initiative, and Professor of Systems Biology at Harvard Medical School.

In principle, confocal microscopes use so-called pinholes to eliminate unwanted out-of-focus fluorescence from image planes above and below the focal plane. By scanning through the sample, plane after plane, researchers can gather the desired fluorescence signals emitted from molecule-bound dyes over the entire depth. Specifically, the MPI/Wyss Institute team developed the technique for “Spinning Disk Confocal” (SDC) microscopes that detect fluorescence signals from an entire plane all at once by sensing them through a rotating disc with multiple pinholes. Moreover, “to achieve 3D super-resolution, we placed an additional lens in the detection path, which allows us to archive sub-diffraction-limited resolution in the third dimension” said first author Florian Schueder, a Graduate Student working with Jungmann who also worked with Yin’s Wyss Institute team as part of his master’s thesis.

“This addition can be easily customized by manufacturers of SDC microscopes; so we basically implement super-resolution microscopy without complex hardware changes to microscopes that are generally available to cell biologists from all venues of biomedical research. The approach thus has the potential to democratize super-resolution imaging of whole cells and tissues,” said Jungmann.

“With this important advance, super-resolution microscopy and DNA-PAINT could become more accessible to biomedical researchers, accelerating our insights into the function of individual molecules and the processes they control within cells,” said Wyss Institute Founding Director Donald Ingber, M.D., Ph.D., who is also the *Judah Folkman Professor of Vascular Biology* at HMS and the Vascular Biology Program at Boston Children’s Hospital, as well as Professor of Bioengineering at Harvard’s John A. Paulson School of Engineering and Applied Sciences (SEAS).

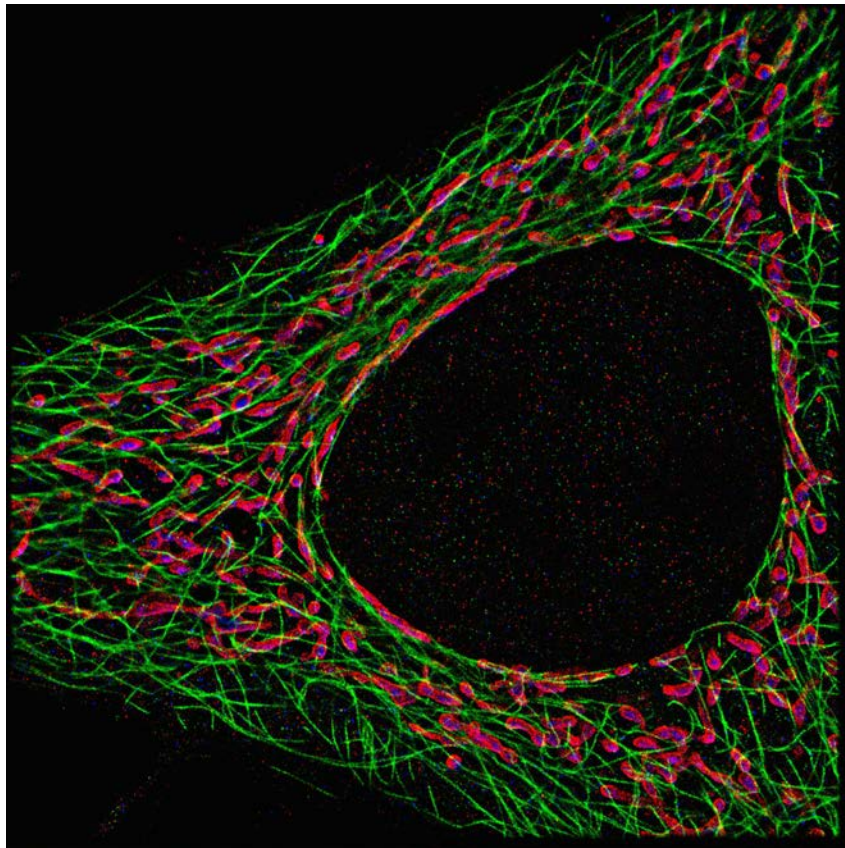
Other authors on the study are past and present members of Yin’s group including Juanita Lara-Gutiérrez; Brian Beliveau, Ph.D.; Sinem Saka, Ph.D.; and Hiroshi Sasaki, Ph.D.; and Johannes Woehrstein, Maximilian Strauss, and Heinrich Grabmayr, Ph.D., who are working with Jungmann. The study was funded by grants from the Wyss Institute for Biologically Inspired Engineering at Harvard University, the German Research Foundation’s Emmy Noether Program, the European





Research Council, LMU's Center for Nanoscience, the Max Planck Society and Max Planck Foundation, the National Institutes of Health and the Office of Naval Research.

By Benjamin Boettner



**Caption:**

Three-color Exchange-PAINT image using a Spinning Disk Confocal Microscope super-resolves the microtubule network (green), TOM20 (red, mitochondrial outer membrane marker), and HSP60 (blue, heat shock protein located in the mitochondrial inner matrix) inside a HeLa cell.

Credit: Florian Schueder, MPI/LMU

**Original publication:**

F. Schueder, J. Lara-Gutiérrez, B.J. Beliveau, S.K. Saka, H.M. Sasaki, J.B. Woehrstein, M.T. Strauss, H. Grabmayr, P.Yin & R. Jungmann: "Multiplexed 3D super-resolution imaging of whole cells using spinning disk confocal microscopy and DNA-PAINT", Nature Communications, December 2017  
DOI: <http://dx.doi.org/10.1038/s41467-017-02028-8>





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## **About Ralf Jungmann**

Ralf Jungmann studied physics at Saarland University in Saarbrücken from 2001 to 2006. After graduating from the University of California Santa Barbara, USA, he earned a doctorate from the Technical University of Munich in 2010. This was followed by a postdoctoral fellowship at the Wyss Institute for Biologically Inspired Engineering at Harvard University. Since 2014, he has been head of the independent Molecular Imaging and Bionanotechnology Research Group at the Max Planck Institute for Biochemistry in Martinsried and Ludwig Maximilian University (LMU) in Munich. He has held a professorship in experimental physics at LMU since 2016. In 2016 Jungmann was awarded the ERC Starting Grant of the European Research Council.

## **About the Max Planck Institute of Biochemistry (MPIB)**

The Max Planck Institute of Biochemistry belongs to the Max Planck Society, an independent, non-profit research organization dedicated to top level basic research. As one of the largest Institutes of the Max Planck Society, 850 employees from 45 nations work here in the field of life sciences. In currently eight departments and about 25 research groups, the scientists contribute to the newest findings in the areas of biochemistry, cell biology, structural biology, biophysics and molecular science. The MPIB in Munich-Martinsried is part of the local life-science-campus where two Max Planck Institutes, a Helmholtz Center, the Gene-Center, several bio-medical faculties of two Munich universities and several biotech-companies are located in close proximity. (<http://biochem.mpg.de>)

## **About the Wyss Institute for Biologically Inspired Engineering at Harvard University**

The Wyss Institute for Biologically Inspired Engineering at Harvard University uses Nature's design principles to develop bioinspired materials and devices that will transform medicine and create a more sustainable world. Wyss researchers are developing innovative new engineering solutions for healthcare, energy, architecture, robotics, and manufacturing that are translated into commercial products and therapies through collaborations with clinical investigators, corporate alliances, and formation of new startups. The Wyss Institute creates transformative technological breakthroughs by engaging in high risk research, and crosses disciplinary and institutional barriers, working as an alliance that includes Harvard's Schools of Medicine, Engineering, Arts & Sciences and Design, and in partnership with Beth Israel Deaconess Medical Center, Brigham and Women's Hospital, Boston Children's Hospital, Dana-Farber Cancer Institute, Massachusetts General Hospital, the University of Massachusetts Medical School, Spaulding Rehabilitation Hospital, Boston University, Tufts University, Charité – Universitätsmedizin Berlin, University of Zurich and Massachusetts Institute of Technology. (<http://wyss.harvard.edu>)





## About the Harvard Medical School

The Harvard Medical School has more than 11,000 faculty working in 10 academic departments located at the School's Boston campus or in hospital-based clinical departments at 15 Harvard-affiliated teaching hospitals and research institutes: Beth Israel Deaconess Medical Center, Boston Children's Hospital, Brigham and Women's Hospital, Cambridge Health Alliance, Dana-Farber Cancer Institute, Harvard Pilgrim Health Care Institute, Hebrew SeniorLife, Joslin Diabetes Center, Judge Baker Children's Center, Massachusetts Eye and Ear/Schepens Eye Research Institute, Massachusetts General Hospital, McLean Hospital, Mount Auburn Hospital, Spaulding Rehabilitation Network and VA Boston Healthcare System.

(<http://hms.harvard.edu>)

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